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A Novel Class of Modified Nucleosides: Synthesis of Alkylidene Isoxazolidinyl Nucleosides Containing Thymine

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The synthesis of hitherto unknown 1-[3-(hydroxymethyl)-2-methyl-4-methyleneisoxazolidin-5-yl]thymine (**5**), 1-{(*Z*)-[3-(hydroxymethyl)-2-methylisoxazolidin-4-ylidene]methyl}-thymine (**6**), and 1-{(*E*)-[3-(hydroxymethyl)-2-methylisoxazolidin-5-ylidene]methyl}thymine (**7**) is described. The first compound can be regarded as a N,O-analogue of Entecavir and DMDC, which contains thymine as a nucleobase. The key allenic nucleobase was prepared in good yields starting from 2,3-dibromopropene. The subsequent 1,3-dipolar cycloaddition proceeded with good site selectivity to give **5** and regioisomeric nucleosides **6** and **7**.

Introduction

Drug discovery for antiviral chemotherapy during the last 30 years has provided effective treatments for numerous viral diseases. In particular, the fight against AIDS has prompted the development of new classes of antiviral drugs, which have provided considerable decrease in HIV-associated mortality as well as improvement in the quality of life of AIDS patients.

Three categories of antiretroviral agents in clinical use are nucleoside reverse transcriptase inhibitors (NRTI),[1] such as zidovudine and stavudine (d4T), protease inhibitors,[2] and nonnucleoside reverse transcriptase inhibitors (NNRTI).[3] Although new, promising targets are being identified, nucleoside analogues remain the cornerstone of antiviral therapy.[4] Currently, seven of the sixteen drugs available for the treatment of AIDS (AZT, ddC, ddI, d4T, 3TC, abacavir, and tenofovir)[5] belong to the NRTI category. Among NRTIs for AIDS, 3TC is also the drug of choice for the treatment of hepatitis B virus infection.[6]

For many years, the design of modified nucleosides has been a focal point of research in medicinal chemistry. Nowadays, among a plethora of synthetic analogues, a number of compounds have emerged that exhibit a broad spectrum of biological activity as the result of their interaction with various pathogen-specific enzyme systems. In this regard, structure–activity relationship (SAR) studies led chemists to design a large variety of modifications of nucleosides that either affect only their base or sugar part or both of these moieties together.[7]

Heterocyclic nucleosides[8] have gained considerable attention during the last years. In this context, our research group has disclosed a new series of extremely modified nucleosides featuring, as a spacer unit, an isoxazolidine system instead of the sugar moiety. These new N,O-nucleoside analogues have shown interesting physiological activities. For instance, (–)-ADFU (**1**) is characterized by low cytotoxicity and, noteworthy, has been shown to specifically induce remarkable levels of apoptosis on lymphoid and monocytoid cells. Phosphonated N,O-nucleosides **2** are proven to be potential antiviral agents (Figure 1).[9] Recently, we have reported the synthesis of azanucleosides; some of which are good anti-HCV inhibitors.

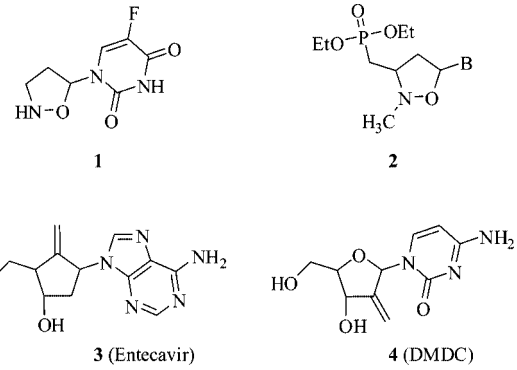

Figure 1. Nucleoside analogues.

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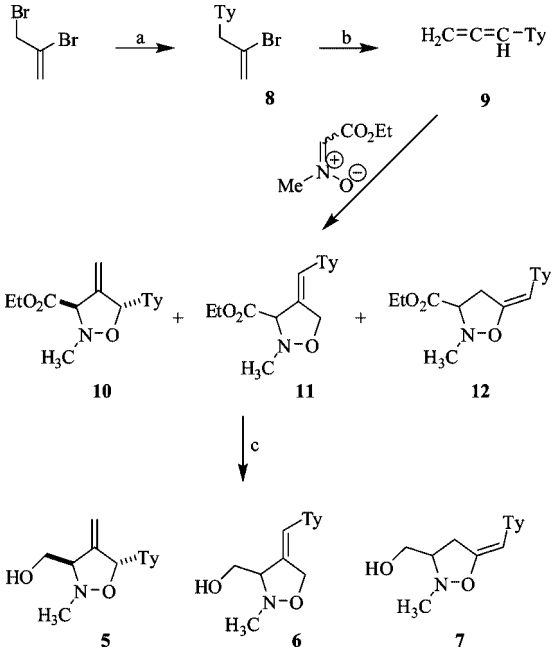
As part of our drug discovery program, and following our interest in isoxazolidinyl nucleosides, we report in this article the synthesis of 1-[3-(hydroxymethyl)-2-methyl-4-methyleneisoxazolidin-5-yl]thymine (**5**) (Scheme 1), the first member of the new series of alkylidene isoxazolidinyl nucleosides. Compound **5** can be considered as a thymine-containing isoxazolidine analogue of Entecavir (BMS-200475) (**3**),[4] which shows potent and selective activity against HBV, and of DMDC (**4**),[10] which is a synthetic analogue of deoxycitidine that potently inhibits the growth of various human tumor cells both in vitro and in vivo.



Scheme 1. a) Silylated thymine, TMSTf, CH_3CN; b) NaOH, dioxane/H_2O; c) $NaBH_4$, dioxane, H_2O.

The designed synthetic methodology allows the simultaneous preparation of two other isomeric analogues **6** and **7** (Scheme 1) with extensively modified carbohydrate units.

Results and Discussion

The synthetic strategy of the present study, which is based on 1,3-dipolar cycloaddition methodology, is depicted in Scheme 1. The key step of the process is the synthesis of the hitherto unknown 1-(propa-1,2-dienyl)thymine (**9**), which was prepared from commercial 2,3-dibromopropene by reaction with thymine in the presence of bis(trimethylsilyl)acetamide. Obtained 1-(2-propenyl)thymine **8** (95% yield) was then treated with NaOH in dioxane/water 1:1 and heated at reflux for 1 h to give target compound **9** in good yield (85%). Subsequent 1,3-dipolar cycloaddition with *C*-ethoxycarbonyl-*N*-methylnitrone heated at reflux in THF for 4 d afforded a mixture of compounds **10**, **11**, and **12** (global yield 30%) in a 2.6:7:1 ratio. Better results were obtained when the 1,3-dipolar cycloaddition process was performed under microwave irradiation: the reaction time was reduced to 4 h and the yields increased to 55%.

The structure of the obtained adducts was assigned on the basis of spectroscopic measurements. Thus, the ^1H NMR spectrum of compound **10** shows the diagnostic resonance of the exocyclic methylene protons at C^4 as two doublets centered at δ = 5.39 and 5.21 ppm (J = 1.2 Hz); the proton at C^5 resonates as a singlet at δ = 5.55 ppm, whereas H^3 gives rise to a singlet at δ = 4.43 ppm.

For compound **11**, the diagnostic resonance of H^6 is at δ = 6.69 ppm, whereas H^3 gives rise to a singlet at δ = 4.85 ppm and the methylene protons at C^5 resonate as two doublets at δ = 4.75 and 4.80 ppm (J = 4.8 Hz). Compound **12** shows the resonance of two methylene protons at C^4 as two distinct doublets of doublets at δ = 2.85 and 3.62 ppm, whereas H^3 resonates as a doublet of doublets at δ = 3.45 ppm.

The assigned stereochemistry of the obtained adducts is supported by NOE measurements. In compound **10**, the lack of NOE for H^3 when H^5 is irradiated is indicative of a *trans* relationship between these protons.

For compound **11**, the (*Z*)-configuration of the double bond was confirmed on the basis of the NOE observed for H^3 when H^6 is irradiated. In **12** on the contrary, no NOE is observed for H^6 when the methylene protons at C^4 are irradiated.

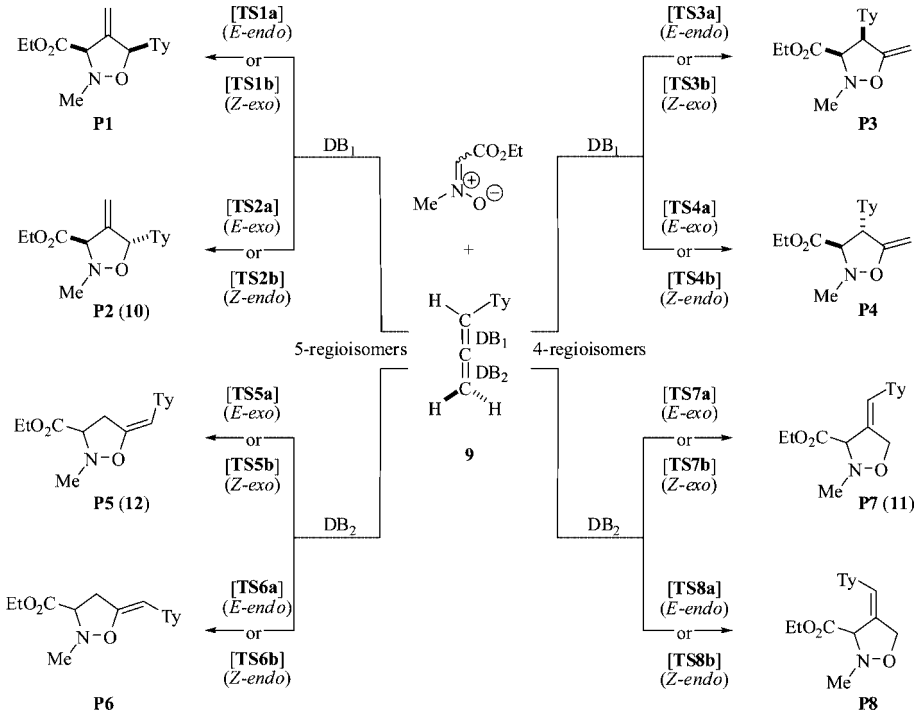
The cycloaddition process shows good site selectivity: attack of the dipole to the unsubstituted double bond of **9** is the preferential reaction course, which gives rise to the formation of **11** and **12** (the ratio of **11/12** versus **10** is 3:1): furthermore, good regioselectivity is also observed with a 7:1 ratio between **11** and **12**.

The cycloaddition process was also rationalized by an *in silico* study with PM3 semiempirical level calculations. To perform this study, we have taken into consideration the eight possible transition states arising from the addition of the nitrone in both the (*E*)- and (*Z*)-configurations to each of the two double bonds (DB1 and DB2) of allene **9** in either an *endo* or *exo* fashion, respectively (Scheme 2). For **TS1**–**4**, the *endo* and *exo* approaches are in accord to the classical nomenclature for Diels–Alder reactions,[11] whereas for **TS5**–**8**, the terminology refers to the thymine moiety that is oriented forward (towards) or backward (away), respectively, from the nitrone functionality along the approach path.

The calculated enthalpy for the formation of all TSs together with the product percentages that were calculated in accordance with the Boltzmann distribution equation are reported in Table 1. These data point out a **10/11/12** ratio of 2.9:7.9:1 that is in good agreement with the experimental one. Moreover, the calculation results showed that compounds **10**–**12** originate from an (*E*)-*exo* TS approach.

The synthetic scheme was completed by reduction of the ethoxycarbonyl group: treatment with $NaBH_4$ afforded nucleosides **5**, **6**, and **7** in 92% yield.

We have devised a slightly different reaction route which allows easy access to minor compound **7**, as the exclusive adduct. In this way, the cycloaddition reaction of *C*-ethoxy-

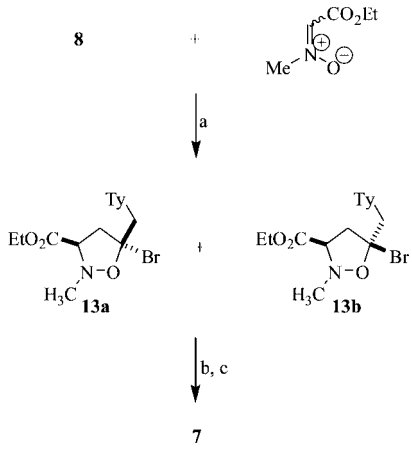


Scheme 2. Combinations of all possible transition states involved in cycloaddition.

Table 1. PM3 formation enthalpies for transition states **1**–**6a**,**b**.

Transition state	ΔH_f	% Calculated	Product (Compound)
TS1a	–64.62	0.70	**P1**
TS1b	–63.29	0.08	
TS2a	–66.73	24.26	**P2** (**10**)
TS2b	–61.50	0.00	
TS3a	–63.23	0.07	**P3**
TS3b	–59.42	0.00	
TS4a	–64.36	0.46	**P4**
TS4b	–60.17	0.00	
TS5a	–66.08	8.15	**P5** (**12**)
TS5b	–63.78	0.17	
TS6a	–63.46	0.10	**P6**
TS6b	–62.35	0.02	
TS7a	–67.31	64.16	**P7** (**11**)
TS7b	–65.19	1.83	
TS8a	–59.91	0.00	**P8**
TS8b	–61.39	0.00	

carbonyl-*N*-methylnitrone with 1-(2-propenyl)thymine **8** led to the formation of derivative **13** as the only obtained adduct, in racemic form, which was subsequently reduced to nucleoside **7** (global yield 52%) (Scheme 3).



Scheme 3. a) THF, microwave at 300 W, 80 °C, 3 h; b) $NaBH_4$, MeOH; c) Et_3N.

Conclusions

On the basis of the consideration that introduction of a rigid structural element into the nucleoside structure can lead to effective antiviral agents,[12] the synthesis of alkylidene isoxazolidinyl nucleosides, hitherto not described in literature, has been designed.

Additional in vitro tests as well as studies of structure–activity relationships are in progress and will be reported at a later date.

Experimental Section

General Section: Melting points were determined with a Kofler apparatus and are uncorrected. Elemental analyses were performed with a Perkin–Elmer elemental analyzer. NMR spectra were recorded with a Varian instrument at 300 or 500 MHz (^{1}H NMR) and at 75 or 125 MHz (^{13}C NMR) with the use of deuteriochloroform or deuterated methanol as the solvent; chemical shifts are given in ppm from TMS as the internal standard. Thin-layer chromatographic separations were performed on Merck silica gel 60-F_{254} precoated aluminium plates. Preparative separations were performed by flash chromatography by using Merck silica gel 0.035–0.070 mm, or by centrifugally accelerated radial thin-layer chromatography (PCAR-TLC) with a Chromatotron Model 7924 T (Harrison Research, Palo Alto, CA, USA); the rotors (2 or 4 mm layer thickness) were coated with silica gel Merck grade type 7749,

TLC grade, with binder and fluorescence indicator (Aldrich 34,644–6), and the eluting solvents were delivered by a pump at a flow-rate of 1.5–3.5 mL min^{-1}.

The identification of samples from different experiments was secured by mixed mps and superimposable NMR spectra.

Preparation of Allylthymine 8: A mixture of thymine (0.05 mol, 6.3 g) and *N*,*O*-bis(trimethylsilyl)acetamide (0.1 mol, 24 mL) in anhydrous acetonitrile (30 mL) was stirred at room temperature until the solution clarified. 2,3-Dibromoprop-1-ene (0.55 mol, 56 mL) and TMSTf (0.005 mol, 0.98 mL) were then added, and the solution was heated at 80 °C for 12 h. The solvent was removed, and the residue was washed with $CHCl_3$. The organic layers were dried with magnesium sulfate and evaporated under reduced pressure to afford the crude product which was purified by flash chromatography ($CHCl_3$/MeOH 99:1). Yield: 1.63 g (95%), white foam. ^{1}H NMR (300 MHz, $CDCl_3$, 27 °C): δ = 1.32 (d, ^3J = 1.2 Hz, 3 H), 4.79 (s, 2 H), 5.71(d, ^3J = 1.5 Hz, 1 H) 5.90 (d, ^3J = 1.5 Hz, 1 H), 7.05 (q, ^3J = 1.2 Hz, 1 H), 9.95 (br. s, 1 H, N–H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 27 °C): δ = 16.0, 54.7, 110.0, 119.5, 120.0, 138.0, 150.8, 163.6 ppm. $C_8H_9BrN_2O_2$ (245.08): calcd. C 39.21, H 3.70, N 11.43; found C 39.10, H 3.68, N 11.46. HRMS: calcd. for $C_8H_9BrN_2O_2$ 243.9847; found 243.9850.

Preparation of Allene 9: A solution of **8** (0.01 mL, 2.45 g) in dioxane (50 mL) was treated with 1 M NaOH (50 mL); and the reaction mixture was heated at reflux for 1 h. After cooling to room temperature, the mixture was evaporated under reduced pressure, and the crude product was purified by PCAR-TLC (hexane/ethyl acetate/Et_3N 5:4.1:0.1; 1.5 mL/min). Yield: 1.55 g (85%), white foam. ^{1}H NMR (300 MHz, $CDCl_3$, 27 °C): δ = 1.95 (d, ^3J = 0.9 Hz, 3 H), 5.56 (d, ^3J = 6.6 Hz, 2 H), 7.02 (q, ^3J = 0.9 Hz, 1 H), 7.23 (t, ^3J = 6.6 Hz, 1 H), 8.95 (br. s, 1 H, N–H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 27 °C): δ = 12.4, 90.2, 96.8, 112.0, 135.6, 138.2, 149.5, 163.7 ppm. $C_8H_8N_2O_2$ (164.16): calcd. C 58.53, H 4.91, N 17.06; found C 58.42, H 4.89, N 17.10. HRMS: calcd. for $C_8H_8N_2O_2$ 164.0586; found 164.0589.

Preparation of Compounds 10–13. Method A: Compound **9** (2.5 mmol, 410 mg) was added to a solution of *C*-ethoxycarbonyl-*N*-methylnitrone (5.1 mmol, 668 mg) in dry THF (20 mL), and the solution was heated at reflux for 4 d. The reaction mixture was then evaporated under reduced pressure and purified by PCAR-TLC (cyclohexane/ethyl acetate 4:1, 3.5 mL/min) to afford compounds **10**, **11**, and **12**, with a global yield of 30% and a 2.6:7:1 ratio. **Method B:** A solution of *C*-ethoxycarbonyl-*N*-methylnitrone (2.6 mmol, 334 mg) and **9** (1.4 mmol, 205 mg) or **8** (1.5 mmol, 395 mg) in THF (8 mL) was irradiated under microwave conditions at 100 W and 80 °C for 4 h. The reaction mixture was evaporated under reduced pressure and purified as in Method A to give compounds **10**, **11**, and **12**, with a global yield of 55% (2.6:7:1 ratio) and compounds **13a**,**b** with a global yield of 30% (1.3:1 ratio), respectively.

Reaction of *C*-Ethoxycarbonyl-*N*-methylnitrone with Allene 9: The first eluted product was ethyl (3*RS*,5*RS*)-2-methyl-5-[5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl]-4-methyleneisoxazolidine-3-carboxylate (**10**). Yield: 55.77 mg, (13.49%), white foam. ^{1}H NMR (300 MHz, $CDCl_3$, 27 °C): δ = 1.35 (t, ^3J = 7.2 Hz, 3 H), 1.90 (d, ^3J = 0.9 Hz, 3 H), 3.25 (s, 3 H, N–CH_3), 4.24 (q, ^3J = 7.2 Hz, 2 H), 4.43 (s, 1 H, 3-H), 5.21 (d, ^3J = 1.2 Hz, 1 H), 5.39 (d, ^3J = 1.2 Hz, 1 H), 5.55 (s, 1 H, 5-H), 7.05 (q, ^3J = 0.9 Hz, 1 H), 8.05 (br. s, 1 H, N–H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 27 °C): δ = 14.1, 15.5, 43.2, 62.2, 75.0, 93.7, 111.0, 111.5, 137.4, 148.5, 150.9, 162.9, 169.0 ppm. $C_{13}H_{17}N_3O_5$ (295.30): calcd. C 52.88, H 5.80, N 14.23; found C 52.95, H 5.83, N 14.19. HRMS: calcd. for $C_{13}H_{17}N_3O_5$ 295.1168; found 295.1173. Second eluted compound was ethyl (3*RS*,4*Z*)-2-methyl-4-{[5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl]methylene}isoxazolidine-3-carboxylate (**11**). Yield: 150.16 mg, (36.32%), white foam. ^{1}H NMR (300 MHz, $CDCl_3$, 27 °C): δ = 1.30 (t, ^3J = 7.2 Hz, 3 H), 1.92 (d, ^3J = 0.9 Hz, 3 H,), 3.21 (s, 3 H, N–CH_3), 4.24 (q, ^3J = 7.2 Hz, 2 H), 4.75 (d, ^3J = 4.8 Hz, 1 H, 5a-H), 4.80 (d, ^3J = 4.8 Hz, 1 H, 5b-H), 4.85 (s, 1 H, 3-H), 6.69 (s, 1 H, 6-H), 7.05 (q, ^3J = 0.9 Hz, 1 H), 8.05 (br. s, 1 H, N–H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 27 °C): δ = 14.2, 15.8, 39.2, 62.2, 70.0, 79.7, 111.3, 117.7, 118.0, 138.4, 146.9, 164.0, 173.1 ppm. $C_{13}H_{17}N_3O_5$ (295.30): calcd. C 52.88, H 5.80, N 14.23; found C 52.83, H 5.81, N 14.27. HRMS: calcd. for $C_{13}H_{17}N_3O_5$ 295.1168; found 295.1164. Third eluted compound was ethyl (3*RS*,5*E*)-2-methyl-5-{[5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl]methylene}isoxazolidine-3-carboxylate (**12**). Yield: 21.45 mg (5.19%), white foam. ^{1}H NMR (300 MHz, $CDCl_3$, 27 °C): δ = 1.32 (t, ^3J = 7.2 Hz, 3 H), 1.92 (d, ^3J = 0.9 Hz, 3 H), 2.85 (dd, ^3J = 7.2 Hz, ^3J = 16.5 Hz, 1 H, 4a-H), 3.23 (s, 3 H, N–CH_3), 3.45 (dd, ^3J = 7.2 Hz, ^3J = 7.5 Hz, 1 H, 3-H), 3.62 (dd, ^3J = 7.5 Hz, ^3J = 16.5 Hz,1 H, 4b-H), 4.24 (q, ^3J = 7.2 Hz, 2 H), 6.65 (s, 1 H, 6-H), 7.12 (q, ^3J = 0.9 Hz, 1 H), 8.10 (br. s, 1 H, N–H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 27 °C): δ = 14.1, 15.7, 35.2, 43.2, 61.2, 72.0, 90.7, 110.3, 133.5, 146.9, 160.8, 162.7, 161.7, 173.1 ppm. $C_{13}H_{17}N_3O_5$ (295.30): calcd. C 52.88, H 5.80, N 14.23; found C 52.75, H 5.78, N 14.20. HRMS: calcd. for $C_{13}H_{17}N_3O_5$ 295.1168; found: 295.1167.

Reaction of *C*-Ethoxycarbonyl-*N*-methylnitrone with Allylthymine 8: The first eluted product was ethyl (3*SR*,5*RS*)-5-bromo-2-methyl-5-{[5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl]methyl}-isoxazolidine-3-carboxylate (**13b**). Yield: 95.93 mg (17%), brown foam. ^{1}H NMR (500 MHz, $CDCl_3$, 27 °C): δ = 1.29 (t, ^3J = 7.2 Hz, 3 H), 1.92 (d, ^3J = 0.9 Hz, 3 H), 2.89 (s, 3 H, N–CH_3), 3.71 (m, 1 H, 4a-H), 4.32 (m, 1 H, 4b-H), 4.33 (q, ^3J = 7.2 Hz, 2 H), 4.78 (d, ^3J = 18.5 Hz, 1 H, 6a-H), 4.91 (m, 1 H, 3-H), 5.16 (d, ^3J = 18.5 Hz, 1 H, 6b-H), 6.88 (q, ^3J = 0.9 Hz, 1 H), 8.80 (br. s, 1 H, N–H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$, 27 °C): δ = 12.7, 14.1, 44.9, 53.0, 54.1, 62.3, 71.4, 82.9, 110.8, 140.6, 150.7, 158.7, 168.1 ppm. $C_{13}H_{18}BrN_3O_5$ (376.21): calcd. C 41.50, H 4.82, N 11.17; found C 41.38, H 4.85, N 11.20. HRMS: calcd. for $C_{13}H_{18}BrN_3O_5$ 375.0430; found 375.0433. Second eluted compound was ethyl (3*SR*,5*SR*)-5-bromo-2-methyl-5-{[5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl]methyl}isoxazolidine-3-carboxylate (**13a**). Yield: 73.36 mg (13%), brown foam. ^{1}H NMR (500 MHz, $CDCl_3$, 27 °C): δ = 1.27 (t, ^3J = 7.2 Hz, 3 H), 1.90 (d, ^3J = 0.9 Hz, 3 H), 2.88 (s, 3 H, N–CH_3), 3.65 (m, 1 H, 4a-H), 4.01 (m, 1 H, 4b-H), 4.31 (q, ^3J = 7.2 Hz, 2 H), 4.83 (d, ^3J = 18.1 Hz, 1 H, 6a-H), 4.88 (m, 1 H, 3-H), 5.22 (d, ^3J = 18.1 Hz, 1 H, 6b-H), 6.81 (q, ^3J = 0.9 Hz, 1 H), 8.92 (br. s, 1 H, N–H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 27 °C): δ = 13.3, 14.7, 45.1, 53.3, 53.9, 64.7, 73.6, 84.5, 112.3, 141.5, 152.4, 159.4, 167.9 ppm. $C_{13}H_{18}BrN_3O_5$ (376.21): calcd. C 41.50, H 4.82, N 11.17; found C 41.62, H 4.83, N 11.14. HRMS: calcd. for $C_{13}H_{18}BrN_3O_5$ 375.0430; found 375.0425.

Preparation of Nucleosides 5–7. General Procedure: $NaBH_4$ (0.1 mmol, 3.8 mg) was added to a solution of isoxazolidine **10**–**12** (0.1 mmol) in dioxane/H_2O (5 mL), and the mixture was stirred for 2 h. The solvent was then removed, and the residue was subjected to column chromatography (chloroform/methanol 95:5) to afford nucleosides **5**–**7**, respectively, in 92% yield.

In the case of isoxazolidines **13**, the crude mixture after the reduction reaction was dissolved in THF (10 mL) and Et_3N (0.1 mmol, 0.014 mL) and heated at reflux for 2 h. The solvent was then removed and the residue was purified as reported above to give **7** in 52% yield.

Reaction of 10 with $NaBH_4$: 1-[(3*RS*,5*RS*)-3-(Hydroxymethyl)-2-methyl-4-methyleneisoxazolidin-5-yl]-5-methylpyrimidine-2,4-(1*H*,3*H*)dione (**5**). Yield: 23.52 mg (92%), white foam. ^{1}H NMR (300 MHz, $CDCl_3$, 27 °C): δ = 1.90 (d, ^3J = 0.9 Hz, 3 H), 2.95 (s, 3 H, N–CH_3), 3.93 (t, ^3J = 5.9 Hz, 1 H, 3-H), 3.95 (dd, ^3J = 5.9 Hz, ^3J = 10.8 Hz, 1 H), 4.04 (dd, ^3J = 5.9 Hz, ^3J = 10.8 Hz, 1 H), 5.15 (d, ^3J = 1.2 Hz, 1 H), 5.30 (d, ^3J = 1.2 Hz, 1 H), 5.95 (s, 1 H, 5-H), 7.05 (q, ^3J = 0.9 Hz, 1 H), 8.05 (br. s, 1 H, N–H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 27 °C): δ = 15.1, 43.1, 57.5, 72.2, 95.7, 110.0, 110.5, 137.4, 148.5, 150.9, 163.8 ppm. $C_{11}H_{15}N_3O_4$ (253.26): calcd. C 52.17, H 5.97, N 16.59; found C 52.05, H 5.95, N 16.62. HRMS: calcd. for $C_{11}H_{15}N_3O_4$ 253.1063; found 253.1065.

Reaction of 11 with $NaBH_4$: 1-{(*Z*)-[(3*RS*)-3-(Hydroxymethyl)-2-methylisoxazolidin-4-ylidene]methyl}-5-methylpyrimidine-2,4-(1*H*,3*H*)dione (**6**). Yield: 23.40 mg (92%), white foam. ^{1}H NMR (300 MHz, $CDCl_3$, 27 °C): δ = 1.95 (d, ^3J = 0.9 Hz, 3 H), 2.98 (s, 3 H, N–CH_3), 3.24 (t, ^3J = 4.8 Hz, 1 H, 3-H), 3.92 (dd, ^3J = 4.8 Hz, ^3J = 10.7 Hz, 1 H), 3.98 (dd, ^3J = 4.8 Hz, ^3J = 10.7 Hz, 1 H), 4.25 (d, ^3J = 6.3 Hz, 1 H, 5a-H), 4.80 (d, ^3J = 6.3 Hz, 1 H, 5b-H), 7.09 (s, 1 H), 7.55 (q, ^3J = 0.9 Hz, 1 H), 9.05 (br. s, 1 H, N–H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 27 °C): δ = 15.6, 43.2, 57.8, 70.6, 74.7, 110.9, 117.3, 117.7, 119.0, 134.9, 146.9, 164.0 ppm. $C_{11}H_{15}N_3O_4$ (253.26): calcd. C 52.17, H 5.97, N 16.59; found C 52.32, H 5.95, N 16.55. HRMS: calcd. for $C_{11}H_{15}N_3O_4$ 253.1063; found 253.1060.

Reaction of 12 with $NaBH_4$: 1-{(*E*)-[(3*RS*)-3-(Hydroxymethyl)-2-methylisoxazolidin-5-ylidene]methyl}-5-methylpyrimidine-2,4-(1*H*,3*H*)dione (**7**). Yield: 23.32 mg (92%), white foam. ^{1}H NMR (300 MHz, $CDCl_3$, 27 °C): δ = 1.93 (d, ^3J = 0.9 Hz, 3 H), 2.05 (dd, ^3J = 3.8 Hz, ^3J = 9.5 Hz, 1 H, 4a-H), 2.30 (dd, ^3J = 4.8 Hz, ^3J = 9.5 Hz, 1 H, 4b-H), 2.68 (s, 3 H, N–CH_3), 2.98 (ddd, ^3J = 3.8 Hz, ^3J = 4.8 Hz, ^3J = 6.5 Hz, 1 H, 3-H), 3.65 (dd, ^3J = 6.5 Hz, ^3J = 10.4 Hz, 1 H), 3.87 (dd, ^3J = 6.5 Hz, ^3J = 10.4 Hz, 1 H), 7.09 (s, 1 H), 7.59 (q, ^3J = 0.9 Hz, 1 H), 9.95 (br. s, 1 H, N–H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 27 °C): δ = 15.6, 43.8, 47.1, 60.6, 69.7, 90.9, 110.9, 134.9, 146.9, 160.6, 163.9 ppm. $C_{11}H_{15}N_3O_4$ (253.26): calcd. C 52.17, H 5.97, N 16.59; found C 51.98, H 5.98, N 16.57. HRMS: calcd. for $C_{11}H_{15}N_3O_4$ 253.1063; found 253.1066.

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